THE BIOLOGICAL EFFECTS OF X-RAYS ON MATING TYPES AND CONJUGATION OF PARAMECIUM BURSARIA

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Introduction

In the field of protozoology one of the most important and spectacular discoveries of recent times is the mating reaction leading to conjugation in *Paramecium* by Sonneborn (1937). If a single specimen of *Paramecium* is isolated and allowed to multiply so that eventually a great number of individuals are produced by vegetative fission alone, the group so derived from the one member is called a clone. Members of such a clone do not mate with each other because they are of one sex type but when one clone is mixed with another clone of the opposite sex type, the mating reaction will occur. In the mixture of the two sex types, the paramecia almost immediately agglutinate or clump together and subsequently conjugate. Upon mixing, a few are first seen to stick together then the clumps become larger and larger until 10–15 minutes later immense clumps of a hundred or so individuals are formed. These large masses then become smaller and smaller until later only small groups of individuals and joined pairs remain. Finally only individual pairs of conjugants (and single ones that were unable to find mates) are left.

Sonneborn discovered and described the mating reaction in *Paramecium aurelia* and Jennings (1938, 1939) described it in *Paramecium bursaria.* One is referred to their original papers for the detailed steps of the phenomenon. Additional information bearing upon the mating reaction and conjugation in *P. bursaria* is given by Wichterman (1944, 1946, 1948).

In an examination of the literature dealing with the biological effects of irradiation with x-rays upon *Paramecium* one is aware of the contradictory reports given by certain of the investigators. Part of the difficulty is due to the fact that earlier workers merely exposed the organisms to the action of x-rays and information upon unit dosage is absent. Without this information, it is impossible to make accurate comparisons of data. At the present time, with modern x-ray generators, it is possible to deliver rays of constant quality and intensity at a given number of roentgen units (*r*) per unit of time.

The main purpose of the present work was to find the effects of roentgen rays upon the mating reaction and conjugation of *Paramecium bursaria.* An abstract of this paper has been published (Wichterman, 1947).

Materials and Method

In the experiments reported here, opposite mating types of *Paramecium bursaria* were used exclusively. *P. bursaria* is the so-called "green *Paramecium*:"

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green because of the fact that living within the cytoplasm of the protozoon are large numbers of extremely small unicellular green algae called zoöchlorellae which fill the body of *Paramecium*. There is thus shown a truly symbiotic existence in the relationship of algae to protozoon.

From eight different clones growing in the laboratory, two of opposite mating type were selected. These, called *C* and *D*, were received from the late Professor Jennings in 1940 and have been cultivated continuously. Organisms of opposite sex type when mixed show a feeble mating reaction in the early morning usually concomitant with the appearance of daylight. The reaction gradually increases in intensity and appears to be strongest at noon. The intensity of the reaction gradually decreases after 1:30 P.M., becoming feeble again at about 4 P.M. The mating reaction leading to conjugation does not occur in the clones under investigation between 4:30 P.M. and 5 A.M. The paramecia are readily cultivated in autoclaved lettuce infusion and grown in glass-covered flasks exposed to light coming from the north. Attempts were made during experimentation to reduce the entrance of bacteria to a minimum but relatively small numbers of bacteria of undetermined species were present which did not interfere with the mating reaction or conjugation. When paramecia are mixed at the proper time, the mating reaction and conjugation have never failed to occur in the seven years the cultures have been under observation.

Paramecia to be irradiated were selected from rich cultures which when tested gave the mating reaction. Concentrated paramecia were then placed in small celluloid boats measuring $4 \times 2\frac{1}{2}$ cm. and containing six cubic centimeters of their original clear culture fluid, then irradiated. Controls were used in every experiment. Paramecia for experimentation and controls were concentrated by simply removing them with a pipette from the upper surface of the cultures where, in daylight, they are found to congregate in great numbers.

The x-ray generator at the Marine Biological Laboratory is one of the most powerful in use for biological experimentation. It operates simultaneously two Coolidge tubes with a current through each of 20 to 30 milliamperes and an alternating voltage of 150 to 200 kilovolts peak. One tube is mounted rigidly near the floor and the other tube is supported on a counter-balanced arm which allows it to be moved vertically and in line directly over the fixed tube. The celluloid boats containing the paramecia to be irradiated were placed between the two tubes and were thus "cross-fired" by the two x-ray beams. Since the tubes can be brought very close together, the intensity of radiation upon the paramecia is extremely high, producing 6100 roentgens per minute. Furthermore, the distribution of radiation is more uniform than would be posible with a single tube. The two Coolidge tubes are water-cooled and since an electric fan was directed on the irradiated material, the slight rise in temperature was negligible during irradiation. Temperature was taken with a small thermometer whose bulb was placed directly in the boats of irradiated paramecia.

General effects of irradiation of Paramecium bursaria with x-rays

It has been known for a long time that paramecia are able to survive exceedingly high dosages of roentgen rays. Irradiation of *P. bursaria* with 100,000 *r* results in greater activity of the organism. They swim faster than the controls, and unlike them do not settle on the bottom of the container.

With dosages of 200,000 *r* and higher, there is a noticeable retarding of motility until at those dosages approaching 900,000 *r* the animals show little movement although the cilia even at 1,000,000 *r* beat erratically. Even at dosages up to 900,000 *r* the paramecia swim in their characteristic manner in the form of a left spiral although in a wobbly fashion.

With dosages of 200,000 *r* and less, the effects of irradiation appear to be lost within a day and all or nearly all of the individuals survive and reproduce. This increase in numbers is seen when irradiated specimens are examined periodically each day for a week or more.

When irradiated with 300,000 *r* the paramecia at first move less actively than the controls but after a day swim actively. At this and lower dosages, the paramecia are capable of trichocyst extrusion when irritated.

An important effect of irradiation with 300,000 *r* and above is the destruction and loss of the zoöchlorellae. Two or three days after irradiation, the paramecia appear paler than before exposure to x-rays and eventually become colorless. Is the colorless condition due to the fact that the paramecia multiply while the zoöchlorellae multiply more slowly? This is hardly the case since roentgen rays inhibit division of the ciliate for a number of days after irradiation. Practically all of the contained zoöchlorellae appear to be destroyed as a result of irradiation but some may persist in a blanched or colorless state. The last green zoöchlorellae to disappear from irradiated specimens are the few that are lodged tightly against the cortex and close to the trichocyst layer.

Single isolations were made from pure-line mass cultures of colorless *P. bursaria* which were bright green with many zoöchlorellae before irradiation. These experimentally produced, colorless specimens have multiplied, resulting in pure-line mass cultures which have the same sex type as before irradiation and which readily mate and conjugate with specimens of the opposite sex type. They are being maintained in permanent culture, are still colorless and are being cultivated in flasks exposed to light in the same manner as the green races.

It is thus possible to obtain races of colorless *P. bursaria* which should prove to be useful in a number of experiments dealing with nutrition and symbiosis, serology, the mating reaction and conjugation. Such x-ray induced colorless forms are capable of living apparently indefinitely even though they change their type of nutrition from a purely holophytic or symbiotic one to a holozoic type in which they must now be dependent upon bacteria in the medium as food. Since it is evident that bacteria are killed in heavily irradiated cultures even though the protozoa are not (Brown, Luck, Sheets and Taylor, 1933), the author inoculated such irradiated cultures of colorless *P. bursaria* with bacteria known to support growth in other colorless races grown in the laboratory. In this manner the paramecia were prevented from starving. Such colorless clones are capable of demonstrating the mating reaction and conjugation with normal green ones of the opposite sex type.

From 400,000 *r* to 1,000,000 *r*, irradiation is seen to result in a decrease in numbers of paramecia. With irradiation of 400,000 *r*, 500,000 *r* and 600,000 *r*, it is possible to obtain survivors and begin new clones all of which are colorless and which later readily mate and conjugate.

Optically active crystals are not found in normal green *P. bursaria* but they are present in other species of *Paramecium*. In x-ray induced colorless *P. bursaria* crystals soon appear in fairly large numbers and the result is similar to that reported by the author (Wichterman, 1941) in which specimens of *P. bursaria* were placed in total darkness for long periods of time. Such specimens when placed in darkness lost most but not all of their zoöchlorellae, and optically active crystals appeared. However, when placed back again in light, the zoöchlorellae soon increased in number and the crystals completely disappeared. With *P. bursaria* irradiated from 300,000 *r* to 600,000 *r,* the green zoöchlorellae disappeared while the crystals became permanent structures even when kept in light.

When irradiated at these higher dosages, the survivors at first show great variation in size from the normal showing dwarfs measuring 64 μ in length and 24 μ in width to giant forms measuring 120 μ in length and 50 μ in width. Survivors of these higher dosages swim forward in a left spiral but in a slow, wobbly manner and appear to be fragile and easily broken. Cyclosis which is more rapid in *P. bursaria* than any other species is barely detectable in the survivors when examined immediately after being irradiated with 600,000 *r*. At this dosage small, clear, structureless vesicles form upon the pellicle which grow to a size of 8 μ, then leave the body. A similar phenomenon was noted by Crowther (1926) in irradiated *Colpidium colpoda,* a ciliate closely related to *Paramecium.*

Irradiation with x-rays markedly increases the viscosity of the protoplasm; higher dosages lead to irreversible coagulation. Survivors that had been irradiated with 600,000 *r* are colorless individuals which later show the normally rapid cyclosis so characteristic of this species. Contractile vacuoles are seen with great clarity and the ciliates possess numerous crystals such as are found in other species of *Paramecium.* At the present time, colorless ones which were green and had been irradiated with 300,000 *r* and 600,000 *r* are flourishing in permanent culture in the laboratory.

One can obtain survivors from clones irradiated with 600,000 *r* and produce rich cultures but above this dosage there are no survivors. When irradiated at dosages of 700,000 *r,* 800,000 *r,* 900,000 *r,* and 1,000,000 *r* the mortality is seen to be exceedingly high soon after irradiation. None survive after the paramecia are transferred to fresh, unirradiated lettuce infusion even when inoculated with bacteria. With 700,000 *r,* paramecia swim very slowly and die in about five hours. With dosages of 800,000 *r* and 900,000 *r* the effect is similar with cytolysis occurring in many. Most specimens die within an hour after irradiation. When irradiated with 1,000,000 *r* and then examined immediately most, but not all, of the animals are seen to be dead. Dead or dying specimens appear swollen or spindle-shaped. The last sign of life that is observed is the movement of cilia which are seen to beat in an erratic manner.

Effect upon mating reaction and conjugation when both sex types are irradiated

Individuals from highly reactive cultures of opposite sex type were irradiated at one time at a given dosage. The dosages ranged from 100,000 *r* to 1,000,000 *r* in steps of 100,000 *r*. The mating types were mixed immediately after irradiation then observed under a low power binocular microscope. The results at given dosages follow and are shown in Table I.

TABLE I

Effect of roentgen rays upon locomotion, the mating reaction and conjugation of Paramecium bursaria

Dosage in roentgens (*r*) of two irradiated mating types of opposite sex (*C* and *D*)	Effect on locomotion	Effect on mating reaction and conjugation		
		Result when two mating types were mixed directly after irradiation	Day following irradiation when mating reaction first occurred	Day following irradiation when conjugation preceded by the mating reaction was seen to occur
100,000 *r*	More active than normal	Mild mating reaction with small clumps of only 10 or less paramecia	1st (immediately followed by conjugation)	1st
200,000 *r*	Slower than normal	Mild mating reaction with small clumps of only 10 or less paramecia	1st (immediately followed by conjugation)	1st
300,000 *r*	Slower than normal	Mild mating reaction with small clumps of only 10 or less paramecia	1st (no conjugation)	2nd
400,000 *r*	Slower than normal	As above but 45 minutes after mixing	3rd, 4th and 5th (no data beyond 5th day)	——
500,000 *r*	Slower than normal	No mating reaction	3rd day, then daily	Few pairs on 7th day and more on following days but most on 12–14th
600,000 *r*	Slower than normal	No mating reaction	3rd day, then daily; clumps increasing in size	Few pairs on 7th day and more on following days but most on 12th–14th
700,000 *r*	Very slow moving	No mating reaction	Never	Never
800,000 *r*	Very slow moving	No mating reaction	Never	Never
900,000 *r*	Very slow moving	No mating reaction	Never	Never
1,000,000 *r*	No locomotion but ciliary activity	No mating reaction	Never	Never

a. Effect of 100,000 r

Upon mixing immediately after irradiation small groups of two to five paramecia were formed which after approximately five hours dispersed into singles again. On the other hand, the controls when mixed at the same time showed huge clumps of a hundred or less individuals which ended in pairs undergoing conjugation. It was not until approximately six hours after irradiation that the paramecia settled characteristically on the bottom of the dish in their normal swimming manner. Apparently the effect of irradiation at this dosage is lost in this time, and all individuals survive. However on the following day there is a normal mating reaction with the formation of large clumps which now culminates in conjugation. It is clear that irradiation at this dosage prevents conjugation on the day of irradiation but does not stop a weak mating reaction from taking place.

b. Effect of 200,000 r

The effect at this dosage is very similar to that mentioned above except that the paramecia before mixing are seen to move less actively than the controls while those irradiated at 100,000 *r* were more active than the controls. When opposite sex types were mixed, small groups of two to five paramecia formed which separated into singles again four hours after irradiation. On the day following irradiation the normal mating reaction occurred which was followed by conjugation.

c. Effect of 300,000 r

When mixed immediately after irradiation, small clusters of paramecia are formed which separate into singles again. The mating reaction again occurs on the day following irradiation but the individuals leave the clumps as before. However on the second day following irradiation, the mating reaction occurs with the formation of larger clumps which now results in conjugation.

In dosages up to 300,000 *r* no appreciable lethal effects were noted. At this dosage and above, animals appeared blanched or pale green several days after irradiation. Later they become colorless. Clones started from isolated colorless individuals regularly demonstrate the typical mating reaction followed by conjugation when mated with normal green *P. bursaria* of the opposite sex type.

d. Effect of 400,000 r

Upon mixing after this dosage there is no immediate clumping but after about 45 minutes, groups form of eight or more individuals. A few small clumps persist for about 10 hours after irradiation which subsequently break down into singles again. This mild clumping reaction occurred daily for the next five days following irradiation but did not result in conjugation during that time. Information is not available beyond this time. At this dosage there is a noticeable decrease in the population of paramecia in the cultures indicating a marked lethal effect. The survivors show little activity while the controls are very vigorous.

e. Effect of 500,000 r

When mixed after irradiation, no clumping of the paramecia occurred; it was not until the third day after irradiation that the mating reaction occurred in which small clumps of two to five individuals were formed. This mild mating reaction then occurred on all subsequent days but did not result in conjugation until a week after irradiation when only a few pairs were present. More pairs formed daily until a maximal number was reached on the thirteenth day. This dosage resulted in an appreciable decrease in numbers of paramecia and also induced great variation in size of specimens.

f. Effect of 600,000 r

No immediate clumping reaction resulted upon mixing but the first appearance of the reaction occurred three days after irradiation. Clumps then consisted of five or less individuals which failed to result in conjugation. After the third day following irradiation clumping occurred daily and did not result in conjugation until on the seventh day when a few conjugants were found. The greatest number appeared on the 12–14th day following irradiation.

Colorless individuals as a result of irradiation at this dosage and at 300,000 *r* are being maintained in permanent culture.

g. Effects of 700,000 r to 1,000,000 r

No mating reaction occurred as a result of these dosages and the animals were seen to move very slowly. Paramecia were unable to survive and all died approximately one to seven hours after irradiation.

Effect upon mating reaction and conjugation when only paramecia of one sex type were irradiated and mixed with normal, unirradiated paramecia of opposite sex type

When paramecia of one sex type are irradiated and mixed with normal, unirradiated paramecia of the opposite sex type, the mating reaction and conjugation occur.

With 300,000 *r* and less, types *C* and *D* of irradiated paramecia produce large clumps when mixed with unirradiated specimens of opposite sex type. These characteristic clumps of the mating reaction are approximately as large as in the controls but require more time to be formed. Similarly, with increased dosages of roentgen units, the lag at which conjugation first appears is lengthened. As an example, those irradiated with 300,000 *r* and mixed with normal specimens of the opposite sex type form clumps of twenty to thirty paramecia in approximately 25 minutes which an hour later develop into clumps of 100 or more specimens. Subsequently, these large clots then break down until 6½ hours after mating only singles are left. On the day following irradiation and mixing, clumping recurs (which in one experiment yielded only one pair firmly joined in conjugation). On the second day clumping recurs which leads into the formation of a number of conjugants but on the third day following irradiation many conjugants are produced after the mating reaction.

After irradiation of one sex type with 400,000 *r* to 700,000 *r*, the paramecia enter into the mating reaction with normal, unirradiated specimens of opposite sex type. The clumps however, are very slow in forming and they are much smaller than in the controls. Thus it requires approximately one hour to form a clump of a dozen or so paramecia when one type is irradiated with 400,000 *r* and there is evidence to show a prolongment of the reaction as noted in the persistence of small clumps to remain long after the animals are mixed. The lag in time in conjugation after the mating reaction increases with the dosage of irradiation until at 700,000 *r* there is a mating reaction which does not lead into conjugation since the irradiated specimens do not survive this dosage.

Result of mixing irradiation-killed paramecia of one sex type with living unirradiated paramecia of opposite sex type

The author (Wichterman, 1940 p. 437) observed and reported the instance of a union resembling conjugating pairs of a living *Paramecium caudatum* with a dead specimen and raised the question of nuclear behavior occurring in the living member.

Boell and Woodruff (1941) reported a specific mating reaction between living *Paramecium calkinsi* of one mating type and a single dead animal of opposite type in their studies dealing with metabolism of mating types. Later Metz (1947) described the induction of "pseudo selfing" and meiosis in *Paramecium aurelia* by formalin killed animals of opposite mating type. He reported that after mixing, clumps of living and dead paramecia broke down in 60–90 minutes releasing single living animals and also pairs of "pseudo selfing" animals. Pairs joined only at the anterior or "holdfast" region and remained united for approximately five hours. "Pseudo selfing" and conjugation animals appeared to have about the same meiotic time table.

Since it has been shown that dead paramecia of one sex type induce clumping with living members of opposite sex type, experiments were undertaken to discover whether *P. bursaria* killed by irradiation would induce the clumping phenomenon with living unirradiated paramecia of the opposite sex type.

Paramecia were killed with dosages of 700,000—1,000,000 *r*, the speed of mortality depending upon the dosage. It should be remembered that generally before the onset of death, cytolysis occurs in many. After irradiation with 900,000 and 1,000,000 *r* dead paramecia were mixed with living members of the opposite type. There was no immediate clumping such as is found in the typical mating reaction. However, a striking phenomenon occurred soon after mixing which at first was strongly suggestive of the mating reaction. Large numbers of living specimens of one sex type clumped around intact dead and disintegrating specimens of the opposite sex type. This appears to be a simple food-reaction such as is found when paramecia congregate around a cluster of bacteria since the phenomenon can be demonstrated when living unirradiated specimens are mixed with irradiation-killed paramecia of the same sex type. Soon after complete cytolysis of dead paramecia, large protoplasmic islands or masses became evident which strongly attracted the living specimens. This did not induce or result in the conjugation of living specimens of the one sex type.

Effects of irradiated culture fluid upon unirradiated P. bursaria

Brown, Luck, Sheets and Taylor (1933) studied the action of x-rays upon the ciliate *Euplotes taylori* as well as the effect of irradiated culture fluid alone on the ciliate. They reported that culture fluid when exposed to x-rays did not produce toxic effects or induce death in *Euplotes*. On the other hand Piffault (1939) reported that irradiation of culture fluid in which *Paramecium aurelia* were swimming may produce secondary or toxic effects which should be taken into account in this type of investigation.

Taylor, Thomas and Brown (1933) found that dosages of 46×10^4 *r* produced death of *Colpidium campylum* within 15 minutes following exposure. They reported that x-radiation with 35×10^4 *r* of a sterile culture medium of 10 per cent yeast extract, tap and distilled water rendered the medium highly toxic or lethal to the ciliates. Their tests showed hydrogen peroxide in the irradiated water in concentrations above 1 : 100,000. They found that de-oxygenated water following the irradiation gave negative tests for H_2O_2 and was not toxic to colpidia. Organic materials such as sheep's blood, agar, gelatin and bacteria, added to the tap water before or after its x-radiation, protected the colpidia against its toxic action. They believed that hydrogen peroxide was in large measure responsible for toxic and lethal effects but suggest that other toxic agents may be produced.

To test the effects of irradiated culture fluid upon unirradiated specimens of *Paramecium bursaria,* experiments were performed in which clear, original culture fluid with paramecia of one sex type (*C*) was irradiated with 1,000,000 *r*, 900,000 *r*, 800,000 *r* and 700,000 *r*. When one cc. of animal-free fluid from these irradiated sets was mixed with three cc. of unirradiated culture fluid containing paramecia of the same or opposite sex type (*D*) no toxic or lethal effects could be distinguished. The same was true when mating type *D* irradiated fluid was added to unirradiated animals of the same sex type or opposite sex type. In daily observations over a period of eight days, there was multiplication of the paramecia with many fission stages evident.

These experiments also disclosed another interesting phenomenon. When paramecia were irradiated at these high dosages and the irradiated animal-free fluid of one sex type was introduced into cultures of unirradiated paramecia of the opposite sex type, clumping of paramecia occurred. The clumps, consisting of a dozen or less paramecia, resembled those characteristic of the mating reaction. The clumps did not lead to selfing or conjugation but broke down into single animals. The fluid lost its ability to induce clumping again when kept for a day and tested. Since the same phenomenon occurred when irradiated, animal-free fluid of one sex type was introduced into cultures of living unirradiated specimens of the same sex type, the clumping is evidently not a mating reaction.

Although not dealing with irradiation studies, Chen (1945) reported that animal-free fluid from a Russian clone of *P. bursaria* induced clumping and conjugation among animals of another mating type even though the latter belonged to a different variety. While the phenomena may be similar to those found in certain algae and other Protozoa, this is probably an example of a "killer" action and not a sex type action.

Effects of irradiation upon joined conjugants of P. bursaria

Experiments were performed in which celluloid boats containing 50 or 100 joined pairs of conjugants were irradiated with 300,000 *r*. Paramecia of opposite mating types and highly reactive were mixed and allowed to form pairs in conjugation. After conjugants were firmly united, pairs were removed with a micropipette and equal numbers then placed into two containers. One container of conjugants represented the controls while the remaining container of conjugants was irradiated 19–20 hours after mating with 300,000 *r*. Both control and ir-

TABLE II

*Length of time control and irradiated conjugants of P. bursaria remained joined together in conjugation process**

Hours after mixing opposite mating types	Number of conjugants separated in controls	Number of conjugants remaining in controls	Number of conjugants separated in irradiated set	Number of living conjugants remaining in irradiated set	Remarks
20	0	50	0	50	
20½	0	50	0	50	
21½	9	41	0	50	
22¼	16	25	0	50	
22½	6	19	0	50	
23	5	14	0	50	
23½	5	9	0	50	
24	3	6	2	48	
24½	6	0	4	44 ←	*Note:* All of controls separated at this time while irradiated conjugants are just beginning to separate.
25	—	—	5	39	
25½	—	—	0	39	
26	—	—	2	37	
26½	—	—	2	35	
27½	—	—	4	31	
28	—	—	2	29	
29	—	—	4	25	
29½	—	—	1	24	
30	—	—	0	24	
30½	—	—	0	24	
31	—	—	0	24	
31½	—	—	0	24	
32	—	—	0	24	
32½	—	—	0	24	
33	—	—	0	24	
33½	—	—	0	24	
42½	—	—	2	22	
43½	—	—	2	20	
44½	—	—	0	20	*Note:* Nineteen irradiated pairs of conjugants died during the process. ↓
45½	—	—	0	20	
47	—	—	1	19	
48	—	—	0	19	
49	—	—	0	18	1 pair died and discarded
53	—	—	0	18	
55	—	—	0	16	2 pairs died and discarded
57½	—	—	0	15	1 pair died and discarded
66	—	—	0	15	
68	—	—	0	14	1 pair died and discarded
70	—	—	0	13	1 pair died and discarded
75	—	—	0	13	
78	—	—	0	11	2 pairs died and discarded
81	—	—	0	4	7 pairs died and discarded
82	—	—	0	0	4 pairs died and discarded

* In this experiment there were used 50 pairs of control conjugants and 50 pairs of conjugants irradiated with 300,000 *r* 19–20 hours after mixing members of each sex. All paramecia of one sex type came from one culture while all members of the opposite sex type came from another culture. Temperature during experiment: 24.5–27° C.

radiated conjugants used in an experiment always came from the same cultures, each of course containing the opposite sex types. The temperature ranged between 24.5–27° C. and was the same for each container at any given time. Conjugants of *P. bursaria* remain joined together in the sexual process for approximately 24 hours at 25° C. (Wichterman, 1946, 1948).

A typical experiment consisting of 50 pairs of control conjugants and 50 pairs of irradiated conjugants is shown in Table II. An examination of the table discloses that all of the control conjugants separated into exconjugants 21½–24½ hours after mating It is at about this time however, that the first of the irradiated conjugants begin to separate. It will be seen from the table that irradiated conjugants separated into exconjugants 24–81 hours after mating.

Of the 50 irradiated conjugants, 19 died during the conjugation process. Irradiated conjugants, like normal ones (as is the case with normal and irradiated single specimens), spiral to the left. Irradiated conjugants, especially those joined for approximately 60 hours and longer, become H-shaped. The paramecia separate along their oral surfaces but are held together by a thick conspicuous protoplasmic bridge in the paroral region. This protoplasmic bridge seems comparable to that described and figured by Sonneborn (1947, p. 286) in the conjugation of *P. aurelia*.

Discussion

In what appears to be an exploratory investigation Bardeen (1906–08) exposed paramecia to x-rays and reported that after a 12 hour exposure, no apparent effect was noted upon the ciliates. He is the first to have observed the effect of x-rays upon conjugants and reported that there was no effect in joined pairs undergoing conjugation or upon their offspring. It is likely that he worked with minimal dosages but this information is lacking from his account.

Schneider (1926), Hance and Clark (1926) and Hance (1931) investigated the effect of x-rays upon vegetative and dividing forms of *Paramecium*. Hance and Clark found the division rate to suffer a slight initial depression lasting two to five days following the exposure. However, the depression was followed by complete recovery. This is in agreement with the author's findings in *Paramecium bursaria* where irradiation was found to temporarily inhibit division. The greater the dosage, the greater the delay in fission. If there is survival of paramecia after exposure to x-rays, the fission rate slowly increases until it is similar to the controls. Indeed, in two of nine isolation cultures in one experiment with *P. bursaria* irradiated with 300,000 *r* and observed daily for 20 days, the division rate was greater than in any of the nine controls.

According to Hance and Clark, dividing specimens showed no different effects than typical vegetative ones when irradiated. They reported that doses repeated at various intervals generally failed to interfere more markedly with the division rate than a single dose. Repeated irradiation caused the paramecia to become slightly swollen without apparent interference with their vitality. According to these investigators, treatments lasting for 10 minutes to three to four hours depressed division and longer or repeated exposures under some conditions raised the reproductive rate.[1]

[1] According to Hance and Clark (1926) the x-rays were produced at 30 kilovolt peak and 22 milliamperes, filtered through very thin cardboard and applied at a target distance of 25.5 cm. Such rays produced about 6×10^{12} pairs of ions per gm. per second in air.

In this regard, Back (1939) irradiated *Paramecium caudatum* to ⅔–⅚ of the lethal dose and found that division of the ciliates could be suspended for several weeks. Accordingly, he considered this lack of division a type of injury inflicted upon the organism.

With *Colpidium colpoda,* a ciliate closely related to *Paramecium,* Crowther (1926) found that a considerable exposure to x-rays produced little visible alteration either in the appearance or motion of the organisms. In an investigation upon the same species, C. Lloyd Claff (personal communication) came to the same conclusion.

Crowther observed that with a given sub-lethal exposure, the ciliates became perceptibly accelerated with rapid and excited appearing behavior followed by a zig-zag motion. When removed at this stage, the majority of ciliates survived. Lethal effects were noted when the forward progression stopped, vacuoles became distended and with the formation of a bubble of clear substance which was extruded from the body, followed by death. It was found that the protoplasm of irradiated *Colpidium* appeared less transparent and distinctly more granular than in normal specimens.

With *P. bursaria,* somewhat similar effects were noted with sub-lethal dosages of 100,000 *r* in that the paramecia became more active in their swimming behavior. Also similar blebs or vacuoles of a clear, structureless substance were formed upon the pellicle of *P. bursaria* after longer irradiation and active cyclosis was considerably decreased or even stopped.

In an inconclusive paper Baldwin (1920) reported the combined action of x-rays and of vital stains upon paramecia but more recently Halberstaedter and Back (1943) observed the effect of the combined action of colchicine and x-rays and colchicine alone upon *Paramecium caudatum.* The concentrations of crystalline colchicine in the basic culture media were 0.0005 per cent, 0.00025 per cent and 0.000125 per cent with the x-ray intensity at the distance of the irradiated object being 80,000 r./m. In colchicine combined with x-rays, the paramecia were maintained for 48 hours in the colchicine solution then transferred to normal culture media for irradiation. They showed that the lethal dose for normal paramecia fluctuated between 200,000 and 700,000 *r,* the half-value doses lying between 300,000 and 250,000 *r.* Their experiments demonstrated that the immediate lethal dose is considerably smaller—about 50 per cent—for paramecia which received treatment earlier with colchicine.

Brown, Luck, Sheets, and Taylor (1933) studied the action of x-rays upon another ciliate, *Euplotes taylori,* in culture fluid and on culture fluid alone. They found that culture fluid exposed to x-rays did not induce death or produce toxic effects on *Euplotes.* The minimal lethal dose to kill the protozoon was found to be 2110 roentgen units per second but much less exposure was sufficient to kill the associated bacteria in the culture fluid. Irradiated bacteria were found to be unsatisfactory as a source of food for *Euplotes.* This difference in resistance to x-ray exposure made it easily possible to sterilize this ciliate. Their important observation adds another method for the sterilization of protozoa and it is very likely that many other species can be sterilized in the same manner.

They found that when *Euplotes* was irradiated for 100–220 seconds there occurred a complete cessation of ciliary activity in many of the organisms, the percentage so affected increasing with the length of exposure. Hours after irradiation such organisms which were seemingly dead regained ciliary movement later.

Later Back and Halberstaeder (1945) made a compreshensive study of the effect of x-rays upon *P. caudatum* and its culture fluid. They found that doses of 100,000 *r* were without visible effects. This is in agreement with the author's findings with *P. bursaria* at this dosage and the paramecia completely recover although they appear excited immediately after irradiation. However, with increased irradiation of paramecia Back and Halberstaeder noted visible alteration of the manner of movement. The change was noted when one-half of the immediate lethal dose was applied. Cilia failed to behave normally and the paramecia swam irregularly backward and forward. With greater dosage, movement ceased and cytolysis occurred. They reported that complete cessation of movement served as an indication of the impending death of the organism and define the immediate lethal dose for *P. caudatum* as that dose which produced complete cessation of motility within 10–15 minutes after irradiation. When 1,000,000 *r* were applied to the culture media alone, no toxic or secondary effect was observed when paramecia were placed in the irradiated fluid. This experiment was repeated by the present author with *P. bursaria* who also found no toxic effect upon the ciliates which is in sharp contrast to the reports of Piffault (1939). It occurs to the author that Piffault may have first killed the bacteria in his irradiated paramecia cultures thereby depriving the ciliates of their source of food. In the experiments reported in the present paper, bacteria known to support growth in other colorless races, were added to the irradiated paramecia. Perhaps the secondary effect reported by Piffault is nothing more than starvation of the paramecia.

Back and Halberstaeder (1945) performed experiments to determine the difference in roentgen-ray susceptibility between immediate (family) and remote (clone) descendents of a single individual. The family represents four to eight descendents of a single organism. Applications of 200,000 *r* caused death in a small percentage of the irradiated population. With increase in the dosage, this percentage rises. At 600,000 *r*, almost all the irradiated specimens died. All survivors of this treatment died after the dose was increased to 700,000 *r*. The dose which produces a 50 per cent mortality rate lies between 350,000 *r* and 400,000 *r*.

They found that when paramecia were irradiated in the same drop, members of a clone died at different roentgen-ray doses. However, under the same conditions, members of a family always died at the same dose.

Concerning tolerance to irradiation of clones within a species, the present work on *Paramecium bursaria* disclosed a difference in the mating types which may only be racial. It was found that members of mating type *C* are more susceptible to x-rays than those of opposite mating type *D*.

It is noteworthy to point out that no one has attempted to make a cytological study of the nuclei or nuclear behavior in irradiated paramecia. Such a study is likely to yield interesting and valuable results.

Summary

1. Individuals of opposite mating type of *Paramecium bursaria* were irradiated with roentgen rays at dosages ranging from 100,000 *r* to 1,000,000 *r* in steps of 100,000 *r*.

2. After 100,000 *r,* locomotion of the paramecia became markedly accelerated but with higher dosages locomotion was retarded. None was able to survive dosages of 700,000 *r* or more.

3. Clones of *P. bursaria* have been established in the laboratory from specimens irradiated with 300,000 *r*–600,000 *r.* One result of such irradiation is the destruction of the contained green symbiotic zoöchlorellae living with in the paramecia. A method is now available for the production of colorless clones of *P. bursaria.*

4. Colorless *P. bursaria* as produced by irradiation have the same sex type as before irradiation. Such specimens readily mate and conjugate with members of the opposite sex type.

5. Members of one mating type are more susceptible to x-rays than those of opposite mating type. This may in reality be only a racial, not a sexual difference.

6. Paramecia of opposite sex types will, when irradiated and mixed, enter into the mating reaction. Clumps thus formed are smaller than in controls and the time taken to form them is greater. The lag in time between conjugation and the mating reaction is dependent upon the dosage.

7. Irradiated conjugants remain joined together in the sexual process much longer than those of the controls.

8. Irradiation inhibits division but the effect is only temporary. After a period of time (depending upon the dosage) survivors have a division rate similar to control specimens.

9. Irradiated culture fluid has no toxic or lethal effect upon unirradiated specimens of *P. bursaria.*

10. Irradiation-killed specimens of one sex type do not mate or conjugate with living, unirradiated members of the opposite sex type nor does it result in selfing.

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